IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Smith et al	§	Confirmation No.: 6303
Serial No.: 10/579,089	§	
Filed: March 9, 2007	§	Group Art Unit: 1789
	§	Examiner: King, Felicia C
For: DAIRY PRODUCT AND PROCESS	§	Attorney Docket No.: DAIRY88.019APC

Statutory Declaration of Stephen Thomas Dybing
Under 37 C. F. R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Stephen Thomas Dybing, citizen of the United States of America,
do solemnly and sincerely hereby declare as follows:

1. I hold a Bachelors degree in Biological Science and a Masters degree in Dairy Science both from South Dakota State University; and a PhD in Food Science from University of Minnesota.

2. A copy of my CV is attached as exhibit "STD1".

3. I am a citizen of the United States of America and have been employed by the New Zealand Dairy Board, the assignee of USSN 10/381,532, (now Fonterra) since 1997 at several US plants and offices.

4. I am currently employed as a Principal Research Technologist and am actively involved in developing new processes for manufacturing dairy and food products, including cheese, ice cream, and butter. I am skilled at making cheese, processed cheese, and related products.

5. In preparation of this Declaration, I reviewed the specification, drawings and pending claims of the above-captioned United States patent application. Additionally, I received and reviewed the final office action (hereinafter "the Action") issued by the United States Patent and Trademark Office (hereinafter "PTO") on 13 April 2011. I also read and studied the previously issued office actions and the previous filed responses as well as the main cited document Johnson et al. (WO 03/069982).

6. I understand that the PTO examiner charged with assessing the patentability of the instant patent application rejected the pending claims as allegedly lacking novelty, being obvious to a person skilled in the art over the main prior art document (Johnston et al.) in view of several other documents cited in the Action.

7. I have read the document Nelson (1970) cited by the examiner in the final office action.

8. The examiner appears to rely on Nelson to support that conclusion that heat inactivation of the flavor producing microorganisms *(Penecillium roqueforti)* is known and further states that it is well known in the art that sterilization is a heat treatment step that kills microorganisms (see the Office Action at page 10).

9. Nelson teaches "inactivation" of the mold, *Penecillium roqueforti* (see abstract). However, looking in more detail at the methodology, Nelson merely sterilizes the mold at 130°C for 4 seconds (see Nelson, page 469, lines 3-5; first column). These conditions are sufficient for sterilization only, i.e., these conditions will prevent further mold growth and will inactivate some of the *Penecillium roqueforti* enzymes. But the stated head conditions will not inactivate all of the mold enzymes.

10. Reaction kinetics provides the recognized, definitive method for the measuring the ability of specified heat treatments to destroy microbes and deactivate enzymes. Indeed, the U.S. Food and Drug Administration (FDA) relies upon reaction kinetics to determine the efficacy of heat treatments in food processing systems. Labuza (2000) in Chapter 2 of Essentials of Functional Foods describes the application of reaction kinetics to food processing, deriving the basic reaction kinetics equations in pages 15 to 36, which are attached as exhibit "STD2". These equations include apparent zero order reactions, apparent first order reactions, the Arrhenius relationship, and the Q_{10} relationship. Many additional references

describing the derivation of these equations and the use of reaction kinetics are readily available.

11. Reaction kinetics is applied to microbial destruction and enzyme denaturation as thermal destruction kinetics. Two major parameters include:
A.) The *decimal reduction time (D)*, or the time required to reduce the microbial population or enzyme concentration by one log cycle (i.e. 90%) at a defined temperature, and
B.) The χ value, or the temperature change required to decrease *D* by 10-times.
Additionally, the F value or "thermal death time" may be used to show the time needed to achieve total microbial inactivation at a specified temperature, i.e. 250°F. Dock and Floros (2000) review the derivation and use of these parameters in determining the affect of heat treatments upon microbes and enzymes in Chapter 3 of Essentials of Functional Foods, pages 49-53 attached as exhibit "STD3".

12. The review of thermal destruction kinetics by Dock and Floros clearly show that temperatures capable of killing all the microorganisms may still be insufficient to destroy all of the available enzymes, as shown in Figure 3-3, Chapter 3 of Essentials of Functional Foods, page 53. It is well known in the art that most oxidative and hydrolytic enzymes (i.e. proteases, lipases, lipoxygenases, amylases, and polyphenoloxidases) present in foodstuffs, survive heat treatment conditions that will inactivate the microorganisms *per se* , i.e. sterilisation conditions. The inactivation of such enzymes requires heat treatments of at least 70-105°C (158 to 221°FD) for several minutes (see page 52, line 17 of exhibit "STD3").

13. If the heating conditions fail to inactivate any flavor producing enzymes, particularly lipases and proteases, then these enzymes will continue to act on a food substance long after the substance has been sterilized. Any continued enzymatic activity produces unacceptable variations in flavor and flavor defects.

14. I attach an extract from a confidential report prepared by inventors Smith and Elston that describes the preparation of camembert/blue cheese (exhibit "STD4"), as evidence of enzyme inactivation. The extract excludes commercially sensitive information.

15. Paragraph 4.2 of the Results section states "The cook temperature of 83°C should have killed all the mould and deactivated any enzymes present". The precise process conditions are set out in paragraph 3.3, namely 83°C for 4 minutes. Paragraph 6.4 of the Discussion section also confirms that "any bacteria and enzymes present in the curd are killed or deactivated and the final product should be very shelf stable".

16. Adding a living flavor producing microorganism to the process of Johnston et al. would not result in enzyme inactivation. The cooking conditions of Johnston et al. were as follows:

- 68°C for 2-4 minutes (examples 1, 2, 16, 17, 18, 19, 20);
- 72°C for 2-4 minutes (examples 3, 4, 5, 6, 7 and 11);
- 62°C for 2-4 minutes (example 8); and
- 65°C for 2-4 minutes (examples 10, 12, 13, 14 and 15).

All of these cooking conditions would result in killing the flavor producing microorganism only (i.e., sterilization). In addition, although these heat treatments would deactivate many of the mold enzymes present, these conditions would not inactive all of the enzymes present.

17. I also point to paragraph 4.4 of the Results section and paragraph 5 Key Findings of the attached Report, which confirms that addition of whey protein to the cheese base resulted in a surprisingly improved flavor. I would not have expected the addition of whey protein to improve the flavour, because intact (i.e. whey proteins that have not experienced proteolysis by proteases) are too large to affect the active sites on human taste buds. Therefore, whey proteins are usually perceived as bland. An improved flavor resulting from whey protein addition is neither disclosed nor suggested in Johnston et al., which does not disclose the addition of whey protein at all, and would not have been expected before the development of the method disclosed in the present application.

I hereby declare that all of the statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the *United States Code* and that such wilful false statements may jeopardise the validity of the application or any patent issued thereon.

AND, FURTHERMORE I MAKE this solemn declaration conscientiously believing the same to be true and by virtue of the Oaths and Declarations Act 1957 of New Zealand.

DECLARED at Palmerston North, NZ) Stephen Thomas Dybing
this 7th day of October) 2011)

BEFORE ME:

VERONICA EATON
SOLICITOR
PALMERSTON NORTH

A Solicitor of the High Court of New Zealand

This is the exhibit marked "**STD1**"
annexed to the Declaration of **Stephen Thomas Dybing** affirmed at
Palmerston North NZ this 7th day of October 2011 before me

VERONICA EATON
SOLICITOR
PALMERSTON NORTH

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A Solicitor of the High Court of New Zealand

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Career Summary

Professional Accomplishments:

- Developed process for increasing cottage cheese yield by addition of sodium hexametaphosphate.
- Prepared ingredient and nutritional labels; screened and selected ingredients (i.e.. cocoa powder), and improved the efficiency of manufacturing procedures for ice cream,
- Developed procedures for producing cheese base (Cheddar cheese for manufacturing, or CCFM) from UF retentates, subsequently tested in process cheese manufacture,
- Lead research team that identified calcium lactate crystals on Cheddar cheese, developing procedures for limiting crystal formation,
- Developed a Sonic cheese manufacturing procedure subsequently patented at Land O'Lakes,
- Contributed to development of manufacture of neutralized starter at cheese plants,
- Developed procedure for manufacturing hard cheese varieties such as Cheddar from UF retentate,
- Taught classes on cheese manufacture, thermodynamics of drying, sensory evaluation of hard grating cheese, and process cheese manufacture to plant personnel, as well as food laboratory analysis to graduate students at University of Minnesota (Chemical and Instrumental Analysis of Foods).
- Modelled lactose crystallization and the evaporation and drying of whey, acid whey, and milk to maximize processing efficiency and minimize manufacturing problems,
- Developed process for producing highly flavoured butter,
- Monitored process cheese manufacture at a plant in Holland for a client U.S. company,
- Established analytical laboratory for U.S. cheese company at new plant.
- Performed QA/QC, prepared customer cheese specifications, and developed procedure for producing enzyme modified cheese,
- Developed procedures for producing cheese from UF retentates subsequently patented,
- Prepared ingredient and nutritional labels, sensory grading system, and computerized operator processing logs for cheese and drying plants.
- Developed thermodynamic model for drying cheese and fluid dairy products.
- Developed computerized mass balance for a cheese plant,

- Developed secondary starter media,
- Prepared and submitted requests for regulatory or freedom to operate opinions from legal council,
- Contributed to major technical customer service projects in U.S., Jamaica, Honduras, and New Zealand,
- Performed numerous proximate and sophisticated analysis of dairy products, particularly in University work,
- Mathematically analyzed cheese manufacture and yield, drying thermodynamics, etc.,
- Extensively worked in laboratories, pilot plants, and processing plants,
- Currently contributing to major projects.
- Active in professional associations, particularly American Dairy Science Association. Acquainted with most of academic colleagues in the U.S. and Canada.

Employment:

Fonterra: 1997 to Present. Currently Principal Research Technologist
Dietrich's Milk Products, Reading, PA. 1995 – 1996. Food Technologist.
University of Minnesota, Dept. Food Sci. and Nutrition. St. Paul, MN.
Research Fellow: 1992
Graduate Assistant 1985-1991
Consultant
Established analytical laboratory for cheese plant in Idaho 1993.
Represented a U.S. food company at a process cheese plant in Holland, 1992.
Land O'Lakes, Inc. Arden Hills, MN.
Scientist: Research and Development: 1979 – 1985 Full time,
Leave of Absence: 1985-1989.
South Dakota State University: Dairy Science Dept., Brookings, SD Graduate Assistant: Responsible for operating Statewide Services Dairy Laboratory: 1977-1978.
Various previous summer jobs: 1971-1976.

Education:

Ph.D. Food Science. 1994. University of Minnesota, Saint Paul. Advisor: David E. Smith
M.S. Dairy Science. 1979. South Dakota State University. Brookings. Advisor John. G. Parsons
B.S. Biological Sciences (minor in Chemistry). 1976. South Dakota State University. Brookings. Advisor: Shirley W. Seas.

Professional Socities:

American Dairy Science Association

Outside Activities:

Eagle Scout, Boy Scout master, Troop 70, Fridley, MN, 1979 to 1988.
President and player coach: South Dakota State University Ice Hockey team. 1978-1979.
Toastmasters

Publication List
Stephen T. Dybing

Papers Published in Scientific Journals.

Dybing, S. T., and D. E. Smith. 1998. The ability of phosphates or κ-carrageenan to coagulate whey proteins and the possible uses of such coagula in cheese manufacture. J. Dairy Sci. 81:309-317.

Dybing, S. T., D. E. Smith, K. F. Eckner, and E. A. Zottola. 1998. Laboratory procedure for manufacturing Cheddar-type cheese varieties from 5x ultrafiltration retentate. Milchwissenschaft 53(7):337-380.

Dybing, S. T., and D. E. Smith. 1991. Relation of chemistry and processing procedures to whey protein functionality: a review. Cult. Dairy Prod. J. 26(1): 4-9, 11 and 12.

Dybing, S. T., J. A. Wiegand, S. A. Brudvig, E. A. Huang, and R. C. Chandan. 1988. Effect of processing variables on the formation of calcium lactate crystals on Cheddar cheese. J. Dairy Sci. 71:1701-1710.

Dybing, S. T., S. A. Brudvig, J. A. Wiegand, and E. A. Huang. 1986. A simple method for estimating the extent of surface crystal development on colored Cheddar cheese. J. Food Prot.. 49:421-422.

Parsons, J. G., S. T. Dybing, D. S. Coder, K. R. Spurgeon, and S. W. Seas. 1985. Acceptability of ice cream made with processed wheys and sodium casein ate. J. Dairy Sci. 68:2880-2885.

Dybing, S. T., J. G. Parsons, J. H. Martin, and K. R. Spurgeon. 1982. Effect of sodium hexametaphosphate on cottage cheese yields. J. Dairy Sci. 65:544-551.

Patents;

US 7,192,619 B2 Mar. 20, 2007. Modified milk protein concentrates and their use in making gels and dairy products. Dybing, S. T., G. V. Bhaskar, F. P. Dunlop, A. M. Fayerman, and M. J Whitton..

See also NZ 511095
WO/2002/082917
US2003/0054068 A1 Mar. 20, 2003

US 6,177,118 B1 Jan. 23, 2001. Methods for producing cheese and cheese products Blazey, N. D., S. T. Dying, R. J. Knights, I-Lo Huang.

See also NZ 511420
WO/2000/027214

US 5,006,349 Apr. 9, 1991. Process for producing a protein product. Dahlstrom, D, S. T. Dybing, and B. J. Gaffney, Assignee: Land O'Lakes, Inc.

Outstanding Published Patent Applications

US 2004/0043124 A1 Mar. 4, 2004. Method for producing a food product from a concentrated protein. Dybing, S.T.
See also WO 2004/017743 A1

Thesis:

Effect of whey protein incorporation into Cheddar cheese using ultrafiltration techniques on product yield, body, and texture (Vol. I & II) PhD Thesis. University of Minnesota. 1994 UMI Order Number 9505397

Effect of sodium hexametaphosphate on cottage cheese yields. MS Thesis. South Dakota State University 1979

Published Abstracts:

Dybing, S. T., and D. E. Smith. 1991. Protein recovery and curd tension in rennet coagulums of 4.8x UF whole milk retentates and treated whey protein concentrates. J. Dairy Sci. [Abstract] 74(Suppl.1):129.

Dybing, S. T., and D. E. Smith. 1989. Yield, body and texture of Cheddar cheese made from milk, 1.5x, or 4.8x UF retentates and set with varying amounts of calf rennet or a 50:50 mixture of calf rennet-porcine pepsin. [Abstract]. J. Dairy Sci. 72(Suppl. 1) 131-132.

Dybing, S. T., and D. E. Smith. 1988. Effect on the yield, body and texture of Cheddar cheese manufactured from 1.5x or 5x UF retentates [Abstract]. J. Dairy Sci. 71(Suppl. 1) 81.

Dybing, S. T., J. G. Parsons, and K. R. Spurgeon. 1979. Effect of sodium hexametaphosphate on cottage cheese yields. [Abstract] J. Dairy Sci. 62(Suppl. 1):49.

This is the exhibit marked "**STD2**"
annexed to the Declaration of **Stephen Thomas Dybing** affirmed at
Palmerston North this 7th day of October 2011 before me

VERONICA EATON
SOLICITOR
PALMERSTON NORTH

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A Solicitor of the High Court of New Zealand

CHAPTER 2

Functional Foods and Dietary Supplements: Product Safety, Good Manufacturing Practice Regulations, and Stability Testing

Theodore P. Labuza

This chapter will address the legal and scientific issues involved in the manufacturing of nutraceuticals/functional foods/dietary supplements with respect to safety, Good Manufacturing Practice regulations (GMPs), and shelf life testing. Since there are many definitions of these products, we need to define them here. Nutraceuticals were originally defined by Dr Steve DeFelice of the Foundation for Innovation in Medicine in 1989 as "any substance that is a food or part of a food that provides medical and/or heath benefits, including prevention and treatment of disease."[1] This is an all-encompassing definition, including foods, medical foods, and dietary supplements, but it has no regulatory basis. This chapter will concentrate on two categories of nutraceuticals, dietary supplements and functional foods, or foods on which a structure-function claim related to health benefits is made because of the presence of some added active ingredient. It should be noted that it is not a legal definition, since none exists in the United States, although functional foods defined as "foods of special dietary use" (FOSHUs) are a legal entity in Japan.

Dietary supplements by law[2] are products in pill, capsule, soft gel, tablet, powder, or liquid form. Dietary supplements may also be in food form but must be labeled as dietary supplements and cannot constitute a whole-meal replacement. There are now many dietary supplements in baked or extruded form resembling real foods such as chips or health bars that are labeled as dietary supplements. Dietary supplements can carry a structure-function claim that relates the value of some active ingredient, hereafter indicated as "X," in terms of its usefulness for health, where "X" can be a metabolite or an herb/botanical product. More importantly, the structure-function claim cannot be a drug claim and must include the following Food and Drug Administration (FDA) disclaimer: "This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease."[3]

As noted, functional foods are a new and undefined regulatory category in the United States. In essence, they are foods that also contain an active ingredient "X" that is either generally recognized as safe (GRAS) or a food additive and for which a structure-function claim is made, similar to one on a dietary supplement. Theoretically and legally, a functional food may also make a health claim as regulated in 21 CFR §101.71. Since a functional food is not a dietary supplement, there seems to be no requirement for an FDA disclaimer statement as there is with a dietary supplement if a structure-function claim is made. Of importance is that the functional food, if marketed as a food, cannot contain a "new dietary ingredient" unless that ingredient is also either GRAS or an approved food additive. The

It is thus logical that in the ANPR for the GMP for dietary supplements,[48] FDA suggested that dietary supplements carry an expiration date. Under 21 CFR §211.66 for prescription and over-the-counter drugs, there is such a requirement. For these products, the date has to be based on the time the concentration decreases to 90% of the label amount in a serving (tablet, capsule, teaspoon, etc), which is in turn based on the lower of the 95% confidence limits for loss of the active ingredient "X." In the ANPR, FDA says that the date must be supported by data, that the manufacturer can use accelerated shelf life testing procedures, and that the date can be adjusted after the product enters the market because of potential effects of different environmental conditions.

Experimental Design

Many factors can influence the rate of deterioration of food products and the active ingredient "X." In general, the rate of deterioration is a function of composition and environmental factors. Composition includes the concentration of the reactive component, catalysts, pH, and water activity (a_w). Environmental factors include temperature, relative humidity, light, and gas composition in the package. The permeability of the package film to oxygen and water vapor is also critical. The factors that influence the deterioration of the product need to be incorporated into the shelf life study. Assuming that the composition of the product is not variable (which is, of course, a question that has not been answered with respect to the presence of "X"), the above environmental factors are the ones that need to be incorporated to enable shelf life prediction. Because some experiments take an extended amount of time (ie, for products with inherently long shelf lives, such as dry tablets and powders), accelerated shelf life testing (ASLT) is often used. The primary application of ASLT for dietary supplements and perhaps functional foods (note that the ANPR for GMPs is for supplements only) is to determine if the functional ingredient "X" maintains an effective dose over its projected distribution time so that an expiration date can be set. ASLT uses higher temperatures or humidity to accelerate the rate of deterioration and then some basic physical chemical principles or a magic number to project what the shelf life would be at normal storage and distribution conditions. One concern in using too high a temperature in testing is that the physical structure of the system can be different at higher temperatures, as in the melting of lipids, the denaturing of proteins, and the glass-rubber transitions of polymeric carbohydrates.[49] In addition, reactions that led to deterioration at high temperatures may be different from those at normal storage conditions so that the projection is false. The different activation energies of the reactions above and below the glass transition could also result in erroneous prediction from high temperature.[50] Other problems of ASLT have been discussed by Labuza and Riboh,[51] Labuza,[52] Labuza and Schmidl,[53] and Taoukis et al.[54]

Kinetic Modeling

Experimental Design Factors

Before we describe the kinetic models, several factors important for the accumulation of kinetic data of good quality and reliability should be mentioned. First, the error in the reaction rate must be reduced by good analytical precision. Benson[55] calculated the theoretical relative maximum error in the rate on the basis of the precision of the method and the extent to which the degradation is carried out. As shown in Table 2–3, the reaction should be carried out beyond 50% change of the initial value so that the proper kinetic model can be selected.

Typically this is done with drugs, even though the shelf life is determined at the 10% change level. With foods, this is generally impossible; the shelf life predictions have greater error, especially when

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Table 2–3 Estimate of Maximum Percent Error in Reaction Rate Constants

Analytical Precision	*Change in reactant species monitored*							
(%)	*1%*	*5%*	*10%*	*15%*	*20%*	*30%*	*40%*	*50%*
0.1	14	2.8	1.4	1.0	0.8	0.6	0.5	0.5
0.5	71	14	7	4.8	3.7	2.7	2.2	2
1.0	>100	28	14	9.7	7.4	5.3	4.4	4
2.0	>100	57	29	19	15	11	8.9	8
5.0	>100	>100	72	48	37	27	22	21
10.0	>100	>100	>100	97	74	53	44	41

based on sensory testing.[56] Importantly, with herbs and botanicals, even though the HPLC procedure for determination of "X" may have a precision error of ±0.1%, the plant material must first be extracted to get "X" out, which can increase the precision error to greater than ±5%. At that level and a 10% end point, the error in predicting shelf life can be as much as 75% to 100%, obviously very unacceptable unless the shelf life is so long that the product would be consumed much before the end point. On the basis of this, Labuza and Kamman[57] have shown that the data should consist of at least eight data points, including zero time, spaced over the 0% to 50% loss, which, when regressed, will yield reasonable confidence limits so that a good prediction of shelf life is possible.

Overall, the loss of "X" should follow the following equation:

$$-d[X]/dt = k_{app}[X]^n$$

where k_{app} is an apparent rate constant and n is the power factor termed the *reaction order*. It should be pointed out that in this expression, the reaction order is not based on true reaction molecularities, so it should be called *apparent* or *pseudo-order*. The value of n is generally set to 0 or 1 for most modes of loss of "X" deterioration, though for some—for example, a free-radical oxidation of a lipid material such as carotenoids—there may be a more complex expression.[58] In complex food systems, the order by which to model kinetic data is usually determined by a variation of the integration method, plotting attribute value X as a function of time (apparent zero order, $n = 0$) and the natural log of attribute value as a function of time (apparent first order, $n = 1$). If the factors for producing good kinetic data discussed previously are employed, then the highest coefficient of determination (r^2) of the linear regression is usually the best apparent order by which to model loss rate and set the shelf life. In 21 CFR §211.66, this is the method of choice for drug expiration dates, and in fact, the regulation allows for zero order to be used, since, as was shown by Labuza and Kamman,[57] the differences between zero and first order up to 50% loss are negligible if the overall precision is good.

Apparent Zero Order

Apparent zero-order kinetic equations in which $n = 0$ have the differential form of

$$-d[X]/dt = k_{obs}[X]_0 = k_{obs}$$

The integrated form of the apparent zero-order expression for a loss of "X" is

$$[X] = [X]_0 + k_{obs}\,t$$

where $[X]_0$ is the initial concentration and $[X]$ is the concentration at some time t. A plot of $[X]$ as a function of time yields a straight line whose slope equals the apparent zero-order rate constant, k_{obs}.

The units of the rate constant are amount of "X" per day or per any time unit used. Linear regression routines available in most spreadsheet programs and statistical packages can then be used to determine the best straight line from the data collected over time, the r^2, and the 95% confidence limits. Figure 2–1 shows a zero-order plot with the lower 95% confidence limit being used to determine expiration date. It must be noted that this is done at constant temperature and moisture content. As noted on the graph, the expiration date based on the 95% confidence limit is less than the value on the regression line for the data. To improve upon this—that is, to narrow the 95% confidence limit—one can take more points over time but the cost of that improvement is generally not worthwhile from a statistical standpoint.[57] Generally, it is better to improve on the analytical method, especially the extraction process used to analyze for "X."

Assuming that the setting of a standard for the expiration date will be less rigorous for dietary supplements and functional foods than it is for drugs, one can surmise that FDA might use the time for

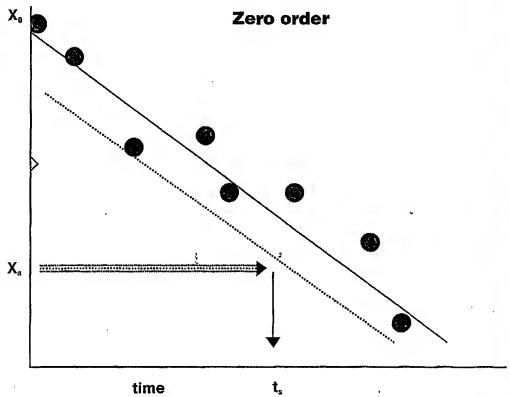

Figure 2–1 Plot of loss of the active ingredient "X" as a pseudo–zero-order reaction with expiration date at t_a. The dotted line represents the lower 95% confidence limit. The expiration date is based on reaching some level of "X" that makes the product ineffective on the basis of its structure-function claim. *Source:* Copyright © 1999, Theodore Labuza.

20% loss, as is done in nutritional labeling for labile vitamins such as Vitamin C. The regulations for label compliance appear in 21 CFR §101.9(g). The 80% retention time for zero-order $t_{0.8}$ is defined as

$$t_{0.8} = 0.8[X]_0/k_{obs}$$

and gives the amount of time for the concentration of $[X]_0$ to be reduced by 20%. Apparent zero-order kinetics have found ample use in modeling the Maillard reaction.[59–61]

Apparent First Order

Apparent first-order kinetics is probably the most widely used kinetic model for food and drug deterioration. The differential of the first-order kinetic expression has the form

$$-d[X]/dt = k_{obs}[X]$$

and the integrated equation for a loss of the quality factor "X" takes the form

$$\ln[X/X_0] = -k_{obs}t$$

Thus, a plot of either $\ln[X]$, $\log[X]$, $\ln[X/X_0]$, or $\log[X/X_0]$ as a function of time gives a straight line with a slope equal to the pseudo–first-order rate constant k_{obs}. Note that log denotes "log" to the base 10, while "ln" represents the natural log. The units of the rate constant are reciprocal time (eg, 1 per day). To make things easier, one can plot the fraction remaining, X/X_0 (or percentage remaining = $100 \times X/X_0$), on semilog graph paper to get a straight line. In this case, the slope must be multiplied by 2.303 to convert to the true k_{obs}. Figure 2–2 shows a zero-order semilog plot, with the lower 95% confidence limit being used to determine the expiration date. It must be noted that the experiment is carried out at constant temperature and moisture content. From a kinetic standpoint, if the data show a straight line on this type of plot for more than two half-lives (ie, <25% remaining), then the order is most likely first order. In drug expiration testing, generally the experiment is carried out to three half-lives (12.5%) remaining with lots of points in order to reduce the 95% confidence limits.[62] As before, assuming that the setting of a standard for the expiration date will be less rigorous than for drugs, one can surmise that FDA might use the time for 20% loss, as is done in nutritional labeling for loss of natural vitamins under 21 CFR §101.9(g).

The time for 20% loss for a first-order model is defined by

$$t_{0.8} = 0.223/k_{obs}$$

Many reactions can be modeled as pseudo–first-order reactions, including vitamin C degradation,[50] aspartame degradation,[63–65] and amino acid or reducing sugar loss due to the nonenzymatic browning.[66]

Effect of Temperature on Shelf Life

The fact that most reaction rates increase with increasing temperature is well recognized. Increased reaction rates because of temperature abuse during distribution and storage are a critical factor in the design of the shelf life testing of both foods and drugs and, for the same reason, of dietary supplements. The effect of temperature is used in the design of ASLT experiments to speed determination of the expiration date. The use of ASLT was recognized in the GMP ANPR with respect to expiration dating of dietary supplements. The influence of temperature on the rate of deterioration has been shown to follow the Arrhenius relationship, which is given by

$$k_{obs} = k_A \exp(-E_a/RT)$$

where k_A is the pre-exponential constant, E_a is the Arrhenius activation energy in Kjoules/mole and is a measure of the temperature sensitivity of the reaction (ie, the higher the value, the faster the reaction

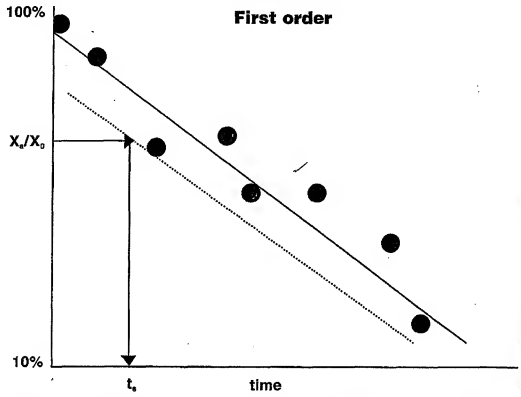

Figure 2–2 Plot of loss of the active ingredient "X" as a pseudo–first-order reaction with expiration date at t_e. The data are plotted on a semilog scale with the value of $XX_0 \times 100$, which is equal to the percentage remaining. The dotted line represents the lower 95% confidence limit. The expiration date is based on reaching some level of XX_0 that makes the product ineffective on the basis of its structure-function claim. *Source:* Copyright © 1999, Theodore Labuza.

as temperature increases), R is the ideal gas constant (1.986 Cal/mol K or ~8 Js/mol K), and T is the temperature in Kelvins. A plot of the natural log of the rate constant from either an apparent zero-order or an apparent first-order reaction as a function of the reciprocal of absolute temperature yields a straight line with a slope of $-E_a/R$, as shown in Figure 2–3. For confidence in the value of the slope for extrapolation to a lower temperature, either rate constants at a minimum of five different temperatures, rate constants at three temperatures analyzed by the point-by-point method, or nonlinear regression of all the data simultaneously from at least two temperatures must be used.[57,67] If only three k values are available (ie, only three test temperatures are used), the confidence range is usually wide since the Student's $t_{\alpha/2}$ value is large. Because of the cost of shelf life testing of foods, especially by sensory analysis, only rarely are more than two temperatures used, but this may be possible for dietary supplements where only "X" is being measured. Figure 2–3 also shows the value of the Arrhenius plot from an accelerated shelf life testing standpoint. If the linear relationship holds, one can use

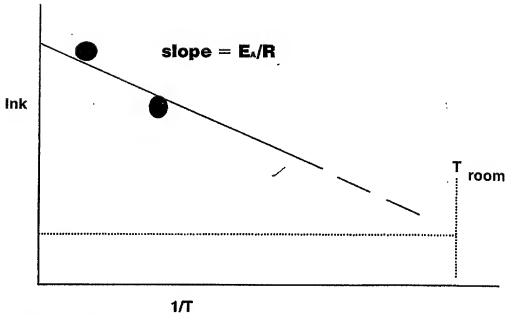

Figure 2–3 Typical Arrhenius plot with extrapolation to room temperature. *Source:* Copyright © 1999, Theodore Labuza.

high-temperature data to extrapolate to lower temperature, the "magic number" approach. Of course, the more temperatures you use, the better the prediction, but the minimum is two to be able to draw a straight line. The magic number, then, is the factor you multiply by the shelf life at the highest temperature of the test to get the shelf life at the presumed storage temperature to which the product will be exposed. Obviously, one needs a minimum of two test temperatures to do this and a reasonable guess as to the conditions of storage and distribution. The latter is not well known even for foods.[68]

Because use of Arrhenius activation energy plots is confusing to many people in terms of understanding natural logs of rate constants and inverse absolute temperature in Kelvin, another method of expressing the temperature dependence of reactions is the Q_{10} approach. The Q_{10} is defined as the ratio of the rate of reaction at one temperature compared to that at a temperature 10°C higher or lower. It can also be defined as the ratio of the shelf life at one temperature to that at a temperature 10°C higher or lower. For example, a Q_{10} of 2 means that if the shelf life at 30°C is 3 months, the shelf life at 40°C is 1 1/2 months, while at 20°C it is 6 months. Plotting the log of the shelf life (using any definition for end point, such as the 20% loss value) as a function of temperature (or shelf life vs temperature on a semi-log plot) yields a straight line with a slope of b, as shown in Figure 2–4. As with the Arrhenius plot, one can extrapolate from high temperature to room temperature, assuming that all factors remain constant, which is not always the case, although this is the basis of ASLT and the magic number.

The Q_{10} is thus expressed by the equation

$$Q_{10} = \exp(10b) = \text{Shelf Life at } T / \text{Shelf Life at } (T + 10)$$

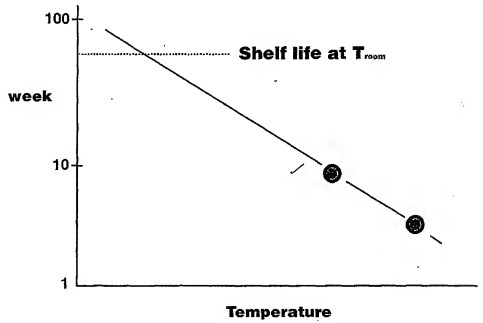

Figure 2–4 Typical shelf life plot with two temperatures, showing extrapolation to room temperature. *Source:* Copyright © 1999, Theodore Labuza.

The Q_{10} and activation energy are related as shown in the following equation, where T is in Kelvin, or degrees Centrigrade plus 273.15:

$$\ln(Q_{10}) = 10E_a/RT(T + 10)$$

The Q_{10}, being temperature dependent, holds constant over a narrow range of temperatures (generally a range of 15–25°), while E_a holds over much larger range; thus, for extrapolating data over large temperature ranges, the Arrhenius equation yields more accurate results. In any case, given the caveats about extrapolation, from the Arrhenius activation energy or the Q_{10} plots, shelf life can be predicted at any temperature.

Figure 2–5 a and b show examples of an Arrhenius plot and shelf life plot for actual components "X" from the food field. These plots are the basis for determining the magic number. It should be made clear that there is no universal number because the activation energy and Q_{10} will vary for each reaction, so at least two temperatures are needed to determine a magic number. Even if you know the Q_{10} range, which is about 2 to 10 for many typical reactions, the extrapolation will give an enormously large range for prediction of potential shelf life, as shown in Figure 2–6. The shelf life of 3 weeks found at 43°C projects to a range of 12 weeks ($Q_{10} = 2$, magic number $= 2 \times 2 = 4$) to almost 4 years ($Q_{10} = 10$, magic number $= 10 \times 10 = 100$) at 23°C. Obviously, one needs a minimum of two temperatures to refine the prediction with the correct Q_{10}. In any case, in the ANPR for dietary supplements, FDA stated that one can adjust the ASLT-determined expiration date after gathering information on actual losses during real-time distribution.

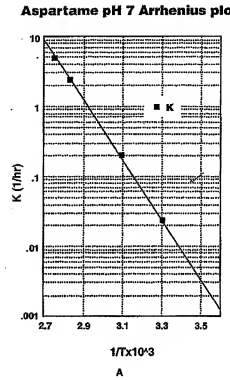

Figure 2–5 Influence of temperature on kinetics. **A,** Arrhenius plot for aspartame degradation in a dry model system at pH 7. **B,** Shelf life plot in time to 25% loss for ascorbic acid in a ready-to-eat cereal at three different moisture contents (water activities). *Source:* Copyright © 1999, Theodore Labuza.

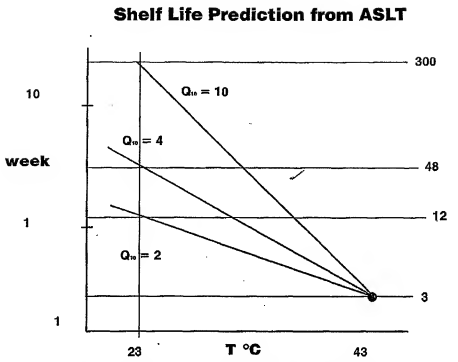

Figure 2–6 Extrapolation of shelf life found at 43°C (ie, using one temperature) to shelf life at room temperature (23°C). The magic number for extrapolation to 23°C is the Q_{10} squared. *Source:* Copyright © 1999, Theodore Labuza.

It also must be made clear that the effect of temperature is critical in processing. Most herb/botanicals are generally dried under mild conditions so that the functional factor "X" is not destroyed. If it is added to a drink that is thermally processed or a cereal dough that undergoes extrusion or baking to form a bar, there are serious concerns about the loss of "X." It would seem that these losses would be much greater than they would be in the formation of a caplet or tablet. No data exist concerning the losses that can occur in processing for the critical substance "X" in most herbs/botanicals.

Effect of Moisture Content and Water Activity on Shelf Life

In the mid-1970s, water activity came to the forefront as a major factor in understanding the control of the deterioration of reduced-moisture and dry foods, drugs, and biological systems.[69,70] It was found that the general modes of deterioration, namely physical and physicochemical modifications, microbiological growth, and both aqueous- and lipid-phase chemical reactions, were all influenced by the thermodynamic availability of water (termed a_w or water activity), as well as by the total moisture content of the system. It is the difference in the chemical potential of water (micrograms in joules per mole) between two systems that results in moisture exchange, and above a certain chemical

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.....
A Solicitor of the High Court of New Zealand

ESSENTIALS OF FUNCTIONAL FOODS

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CHAPTER 3

Thermal and Nonthermal Preservation Methods

L. Lotte Dock and John D. Floros

In the past, the only purpose of food preservation was to achieve safety and extend the shelf life of food products. In the last two decades, however, more attention has been given to maintaining the freshlike characteristics of certain foods; retaining important organoleptic attributes such as color, texture, taste, and appearance; and improving foods' functional properties. With the recent introduction of functional foods and nutraceuticals in the marketplace, the retention of nutrients such as vitamins, the bioavailability of functional food components such as antioxidants, and the preservation of other beneficial food compounds have become increasingly important.

To obtain high retention of heat-sensitive nutrients and functional components, reduced thermal processes or nonthermal processes are being sought out. Terms such as *minimally processed* and *freshlike* have become commonplace in food industry and consumer terminology in the 1990s.[1] Manvell[2] defined *minimal processing* as the least possible treatment to achieve a purpose, such as distributing the food safely under specified storage conditions. Under this definition, minimal processing does not specify the quality to be retained. Therefore, a more appropriate term that covers both safety and quality would be *optimal processing*. Optimal processing would thus be defined as the process needed to obtain safe products with the highest possible quality.

This chapter discusses thermal and nonthermal preservation methods that are appropriate for processing functional foods.

THERMAL PRESERVATION METHODS

Conventional Thermal Processing

Conventional thermal processes, such as cooking, blanching, pasteurization, and sterilization have different objectives. Cooking aims at producing a more palatable food, blanching is primarily aimed at destroying enzymes, pasteurization is aimed at destroying some but not all vegetative cells, and sterilization is aimed at destroying all microorganisms.[3] Sterilization used for food processing prevents growth of microorganisms at some expected storage conditions, so the term *commercial sterility* should be used.[3] For example, vegetables meant for frozen storage are only blanched, and conventionally pasteurized milk, yogurt, and other functional foods containing pre- and probiotics must be refrigerated. Thermal treatments needed to produce commercially sterile and shelf-stable foods, such as infant formulas and some weight control products (eg, Slim Fast and Nestle's Sweet Success), depend on product characteristics (eg, pH, viscosity, and water activity), product type, heat resistance and initial load of target microorganism(s), and heat transfer characteristics of the food.

Target Organisms

The amount of heat needed for commercial sterilization depends on the organisms that survive and grow in the product. Most sterile containers are hermetically sealed and contain low oxygen concentrations that inhibit the growth of aerobic microorganisms. Hence, the microorganisms of concern are the anaerobes or facultative anaerobes. Spore-forming organisms are of particular concern because spores are more heat resistant than vegetative cells. Consequently, anaerobic spore-forming pathogens that produce lethal toxins set the constraints needed for heat sterilization, and in many cases, the organism of concern is *Clostridium botulinum*. Products with a pH higher than 4.6 (low acid) must receive a treatment resulting in a 12-log reduction of *C. botulinum*. At pH 4.6 or lower, *C. botulinum* does not grow, and for these products microbial destruction is based on other target microorganisms. Many other organisms are more heat resistant than *C. botulinum* and are used to calculate the extent of thermal treatments. For example, another *Clostridium* species, *C. sporogenes* which is nonitoxic, is used to determine safe thermal processes for low-acid foods.[3] Another anaerobic spore former, *Bacillus stearothermophilus*, is more heat resistant than *C. botulinum*, but its optimal growth temperature is about 49 to 55°C, and it does not often grow at temperatures below 37°C.[3] Therefore, a product containing spores of this organism should be considered commercially sterile when stored at temperatures below 43°C (110°F).

Processing Parameters

Thermal destruction of microorganisms, most nutrients, enzymes, and other quality factors such as flavor, color, and texture obeys first-order reaction kinetics.[3] This means that the reaction rate depends on the concentration of the substance of interest. Two parameters are important in thermal destruction kinetics: (1) the destruction rate at a given temperature and (2) the relationship of destruction rate to temperature. The first parameter is known as the *decimal reduction time* (*D*) and is defined as the time it takes to reduce the concentration by one log cycle, or, in other words, the time to produce a 90% reduction in concentration. In depictions of concentration versus time, *D* is determined by the negative reciprocal of the slope (Figure 3–1A).

The second parameter is known as the z value and describes the relationship of *D* to temperature. When *D* values are depicted versus temperature, z is the negative reciprocal of the slope (Figure 3–1B). In other

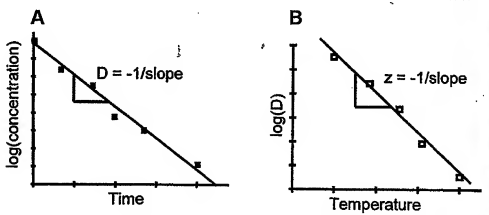

Figure 3–1 Calculation of *D* values from thermal death curves (A) and calculation of *z* values from *D*-versus-temperature plot (B).

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words, z is the change in temperature needed to change D by a factor of 10. If z is small, the microbe or reaction is sensitive to temperature change, and only a small temperature increase is needed to decrease D by 10-fold. On the other hand, if z is big, the temperature change needed to change D 10-fold is big, and the microbe or reaction is less influenced by temperature. Sometimes, the total time to achieve microbial inactivation is given by the so-called F value or thermal death time. The most commonly used F value is the F_{250}, where 250 denotes the processing temperature in degrees Fahrenheit. For low-acid foods, $F = 12D$ corresponding to a 12-log reduction of the target microorganism(s), whereas for high-acid foods $F = 6D$. Another method to measure substance deterioration is the Arrhenius equation:

$$k = A \cdot e^{\frac{-E_a}{RT}}$$

where k is the reaction rate (min^{-1}), A is a constant (min^{-1}), E_a is the activation energy (cal/mol), R is the universal gas constant (1.987 cal/mol × K), and T is the absolute temperature (°K). E_a describes the energy needed to get 1 mol of substance into the active state. The Arrhenius equation is often used in reaction kinetics theory, while the thermal death time method is used for microbial death time studies. However, using D and z is the basis of thermal process calculations and is the most common method used in industry.[3]

Factors Influencing Thermal Resistance of Microorganisms

Factors affecting the thermal resistance of microorganisms can be divided into three major categories: (1) inherent resistance, (2) environmental factors during formation of cells or spores, and (3) environmental factors during thermal treatment.[3] The inherent heat resistance of different strains of the same bacteria can vary grossly. For example, the aciduric strain of *Escherichia coli*, *E. coli* O157:H7, has increased heat resistance in low-pH foods as compared to other nonaciduric strains of *E. coli*. Several factors influence the heat resistance of cells and spores during their growth. For example, spores produced at higher temperatures are more heat resistant than spores produced at lower temperatures.[3] Other factors that may affect heat resistance are ionic strength, organic compounds, lipids, and age of cells. For the latter, the most common belief is that heat resistance is lower during the logarithmic phase and then increases during the stationary phase. Environmental factors that affect heat resistance during thermal treatment include pH, buffers, ionic environment, and composition of the medium. An important factor to consider at all times is water activity, a_w. Generally, spores and cells cannot grow at a_w lower than 0.85, so in dry and semidry foods there is little concern for bacterial growth. However, molds and yeast grow at low a_w and become major spoilage and pathogenic (ie, toxins produced by molds) concerns in dry and semidry foods.

Thermal Degradation of Food Substances

Destruction of pathogenic microorganisms to achieve a high level of food safety is the main objective of thermal processing. However, retention of quality attributes such as flavor, color, texture, and nutritional value must also be considered. Thermal processing may adversely affect some food constituents. In cases where retention of specific nutrients and functional food components is required, their thermal destruction kinetics must be determined, and their dependence on temperature and other environmental factors must be calculated. In certain cases, thermal processing produces positive effects on functional ingredients. For example, lycopene, a carotenoid found primarily in tomatoes and suspected to lower the risk of several types of cancer, becomes more bioavailable when tomatoes are processed. Therefore, lycopene absorption increases 2.5 times in tomato paste as compared to the absorption from raw tomatoes.[4] Similarly, heating blueberries by a mild steam treatment increases the levels of available antioxidants such as anthocyanins.[5] Anthocyanins are flavonoids, functional sub-

stances found in many fruits, specific to blueberries. In another case, processed wheat bran showed an enhanced ability to reduce the development of cancer-related cells as compared to nonprocessed bran.[5] Last, phytochemicals present in rice were more active in rice bran oil rather than in rice bran, implying that processing improves the health-promoting action of functional ingredients such as phytochemicals, probably by increasing their bioavailability.[5]

Denaturation of Proteins. Food proteins are denatured by mild heat, 60 to 90°C, resulting in insolubility and decreased functional properties. However, protein denaturation also improves digestibility and biological availability of essential amino acids. For example, several raw plant proteins exhibit poor digestibility, but moderate heating improves their digestibility without toxin production.[6] In other cases, plant proteins contain antinutritional factors. For example, trypsin and chymotrypsin inhibitors that are present in plant protein impair efficient digestion. Similarly, lectins agglutinate red blood cells, impair protein digestion, and cause malabsorption of other nutrients.[6] Fortunately, both protease inhibitors and lectins are thermolabile. Moderate heat also inactivates proteinaceous toxins such as the *Clostridium botulinum* toxin, which is deactivated at 100°C.[6]

Enzyme Inactivation. Most oxidative and hydrolytic enzymes (ie, proteases, lipases, lipoxygenases, amylases, and polyphenoloxidase) are inactivated by moderate heat treatments. The treatment, termed *blanching*, consists of heating the product to about 70 to 105°C and holding it there for several minutes. *D* and *z* values can describe inactivation of enzymes, but often the activation energy method is used (described in more detail below in the section "Aseptic Processing"). The *D* and *z* values for enzyme inactivation are of the same order of magnitude as those for bacteria. However, some heat-resistant isozymes exist, which may cause off flavors and colors.[3] The target is usually the most heat-stable enzyme that causes quality deterioration during storage. In most cases, this means either peroxidase, lipoxygenase, or catalase. Peroxidase is the most frequently used because its activity is easy to measure and it is the most heat stable. However, peroxidase is not directly involved in quality deterioration of unblanched products. On the contrary, the quality of blanched and frozen products is better if some peroxidase activity remains.[7] Therefore, the best quality products may be obtained if some peroxidase activity remains, the amount of which depends on the product.[7] To determine the optimal point at which peroxidase activity should remain in the product, all possible factors (flavor, color, texture, etc) must be considered at once, and an optimization should be performed. Studies on green beans showed that methods detecting inactivation of catalase, lipoxygenase, and pectin methylesterase, rather than peroxidase, were better ways to prevent quality deterioration.[8] Similar results were found for green peas.[9]

Vitamin Losses. Vitamin losses are not caused solely by processing. Instead, loss of vitamins starts from the time of harvest for most foods,[10] and it is important to consider the loss of specific vitamins. For example, if a food is a major source of a specific vitamin, or a claim is made, then the food processor must maintain and prove the specific vitamin's bioavailability and concentration. Inactivation of enzymes often has a stabilizing effect on vitamins during storage. Heat is not the primary factor of vitamin loss. Instead, losses occur primarily by oxidation and leaching (aqueous extraction).[10] The extent of thermally induced vitamin loss depends on the chemical nature of the food (eg, pH, a_w, dissolved oxygen) and the likelihood of leaching.[10] High-temperature-short-time (HTST) processing is very effective in increasing retention of heat-labile vitamins and other nutrients. The reason is that the *z* values for nutrients and quality factors (eg, color, flavor) generally are larger than those for microorganisms and heat-labile enzymes.[10] Therefore, HTST has a greater effect on microorganisms than on most nutrients. For example, when the temperature is increased 10°C, the reduction in the number of microbial survivors is considerably larger compared to the degradative effect on colors[11] (Figure 3–2). Likewise, *D* values are much greater for nutrients than for microorganisms[11] (Figure 3–3). This allows thermal processes to be optimized for maximum quality retention while achieving the appropriate microbial reduction.

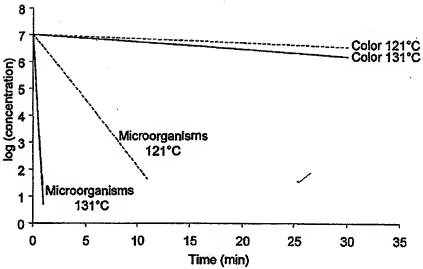

Figure 3–2 The effect of temperature on microbial survivors and color retention. *Source:* Based on D and Z values from P. Jelen, Review of Basic Technical Principles and Current Research in UHT Processing of Foods, *Canadian Institute of Food Science and Technology Journal*, Vol. 16, No. 3, pp. 159–166, © 1983.

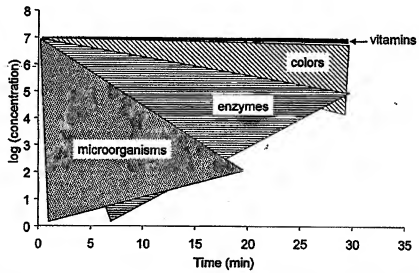

Figure 3–3 Destruction of vitamins, colors, enzymes, and microorganisms during thermal heating at 121°C. The shaded areas indicate the range of *D* values for each of the quality characteristics or inactivation of microorganisms. *Source:* Based on D and Z values from P. Jelen, Review of Basic Technical Principles and Current Research in UHT Processing of Foods, *Canadian Institute of Food Science and Technology Journal*, Vol. 16, No. 3, pp. 159–166, © 1983.

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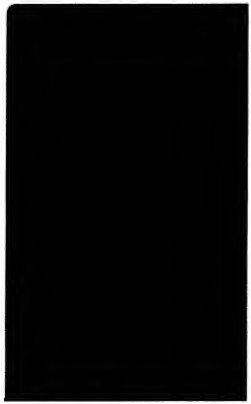

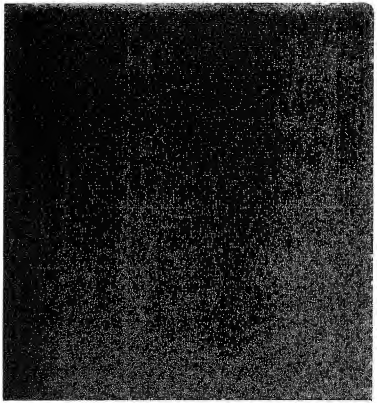

Confidential

Camembert and Blue Processed Cheese Manufacture

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Fonterra Research Centre

Fonterra

Table of Contents

1 Introduction

A mould-ripened Camembert cheese type is produced throughout the world, and in New Zealand specifically for the local market.

Traditionally, Camembert cheese is produced by the addition of both rennet and starter culture to the milk, followed by the processing of that milk into cheese curd. Next, at around 45–52% moisture, the block of curd is placed on racks (10°C coolroom/100% humidity) and spray coated with *Penicillium candidum* for mould growth. This cheese is then held for at least 21 days before final packing and storage. The cheese is expensive to produce and has a limited shelf life, and it is difficult to achieve the right flavour attributes; however, the profitability in terms of monetary returns is good.

This report describes an alternative method of producing Camembert or similar related mould-ripened cheese types. The cheeses made by this method are cheaper to produce, give more consistent flavours and are freeze/thaw stable. Also, it should be possible to shred the Camembert cheese and to land that cheese in excellent condition at any overseas destination for sale months after manufacture. These features are not possible with traditional Camembert cheese types.

Refer to the memo in Appendix 1, giving data on Camembert cheese manufacture.

2 Objectives

- To assess whether desirable flavour compounds (three types A, B, C) can be incorporated into a processed cheese base to produce a Camembert flavour.
- To monitor the flavour development of the added flavour compound over a period of time at a storage temperature of 5°C.
- To investigate whether flavoured Camembert cheese (processed cheese base) can be frozen and re-thawed without affecting the physical structure of the curd base.

3 Experimental

3.1 Manufacture of Curd Base

3.1.1 Medium Fat Base Manufacture

- 40% fat cream was homogenised with pasteurised skim milk at 63°C. A ratio of 1 L of cream to 1 L of skim milk, equivalent to approximately 10% of the total set milk volume, was homogenised; that is, 90% of the skim milk in the silo was not homogenised at all.
- Rennet was added to the cool (10°C) milk mixture at 1 L of rennet to 18,000 L of milk (approximately 3% fat).
- The mixture was held overnight and processed in the morning. Note: the mixture could be processed within 3 h of rennet addition if so desired.

3.1.2 Low Fat Skim Milk Cheese Manufacture

It was also possible to manufacture a low fat skim milk cheese block (no homogenisation or added cream) and to add the fat (high fat cream, 80% fat) to the curd in the kettle to obtain the final fat requirements.

The low fat and medium fat curds were processed as follows.

3.1.3 Precipitation and Cooking

The renneted milk mixture was pumped from the silo and injected in-line with dilute sulphuric acid, to obtain a pH around 5.4. This mixture was then cooked (using direct steam injection) to a temperature of 45°C and held for 50 s in a holding tube. The cooked curd/whey mixture was then either screen or decanter dewheyed.

3.1.4 Washing and Dewatering

The dewheyed curd was then washed in water (pH 2.6 at 36–38°C for 10 min) before being pumped through the decanter for final dewatering. The separated curd could be processed directly from the plant into the kettle or salted and stored as a cheese block to await processing.

3.2 Processed Cheese Production

The skim milk cheese curd blocks produced in the pilot plant were cut into rectangular blocks of 50 mm x 50 mm x 250 mm using a stainless steel cutting wire. These cheese segments were then passed through an Urschel Comitrol Mill Cutter (Model 3600, equipped with a 6 mm head (Urschel Laboratories Inc., Valparaiso, Indiana, USA)) for particle sizing (3 mm) prior to addition to the processing kettle. Both skim milk cheese curds milled well.

3.3 Kettle Processing of the Curd

Using compositional information from the curd base, emulsifying salts, high fat cream, water and citric acid were added to achieve our targets. The curd base was placed in the processing kettle along with the required portion of high fat cream, salt and the mould flavours required. This was followed by the emulsifying salts, citric acid and finally water. Twin augers worked the mixture over itself as the kettle was heated to 83°C over 4 min. The cheese mixture was then dropped out from the base of the kettle into a large scoop, and then

used to fill large shallow plastic sample trays and 225 g butter punnets. The cheese was left overnight in a 5°C room to solidify.

The processed cheese was manufactured (see Appendix 2 for curd recipes) to achieve the following cheese composition:

Composition	
Fat	20%
Protein	21%
Moisture	52%
pH	5.6

3.4 Mould Development in Storage

The following day, the surfaces of the cheeses requiring mould were sprayed with a solution containing 1 g *Penicillium candidum*/500 mL. The cheeses were left in the mould-growing room at 12°C and 100% relative humidity. After 5 days, the mould growth on the cheese was complete and the cheese was turned over and sprayed on the remaining side. After the mould had grown on both sides of the cheese, the cheese was cut into small blocks, flushed with nitrogen and vacuum packed. The cheese was then stored in a 5°C room.

During the 10 days of mould growth, the cheese in trays dried out more rapidly than that in punnets (because of the increased surface area exposed) and would account for the increased stickiness noticed in the punnet cheeses.

Duplicate samples of these cheeses were deep frozen (–18°C) to test their freeze/thaw characteristics.

3.5 Base Flavours

The following flavour types and methods were used to produce the various flavoured cheeses.

3.5.1 Flavour A

Blue flavoured – *Penicillium roqueforti* at 5% w/w was added directly to the processing kettle. *Penicillium candidum* mould was then sprayed on the exterior of the final cheese.

3.5.2 Flavour B

Mushroom flavoured – 2% w/w *Penicillium roqueforti* and 3% w/w *Penicillium candidum* were added directly to the processing kettle. The cheese was packed without mould being sprayed on the outside.

3.5.3 Flavour C

A combination of 2% w/w *Penicillium roqueforti*, 3 mL of esters prepared from cream (sweet flavours) and 25 mL of peachy flavour (MsAji) was added to the processing kettle. *Penicillium candidum* was also sprayed on the outside of the cheese.

3.5.4 Flavour D

In addition to the three flavoured cheeses described above, two further cheeses were manufactured:

1 a control cheese, with mould sprayed on the exterior;

2 a control cheese with 20% (w/w) whey protein powder (ALACEN 392) added into the processing kettle.

Both of these processed cheese samples were cooled to 12°C and sprayed on the outside with *Penicillium candidum* mould. They were then left for 2 weeks, before being placed in a 5°C chiller room.

3.6 Cheese Analysis

The kettle-processed cheeses were stored at 12°C before being coated with mould. These cheeses were then analysed for both composition and microbiological quality (see Appendix 3, Tables A3.1 and A3.2).

3.6.1 Compositional Analysis

Moisture	NZTM3	12.6 (NZMP Wellington)
Fat	NZTM3	6.4 (NZMP Wellington)
Protein	NZTM3	15.8 (NZMP Wellington)
Calcium	NZTM3	16.7 (ICP-OES) (NZMP Wellington)
Salt	NZTM3	9.9 (NZMP Wellington)
pH	NZTM3	2.8 (NZMP Wellington)

3.6.2 Microbiological Analysis

Clostridium perfringens count/g	NZTM2	46.1 (NZMP Wellington)
Coliform detection count/g	NZTM2	48.3 (NZMP Wellington)
Coliform count/g	NZTM2	48.1 (NZMP Wellington)
Escherichia coli/g	NZTM2	48.5 (NZMP Wellington)
Listeria detection (IDF method)/25g	NZTM2	53.2 (NZMP Wellington)
Coagulase-positive staphylococci/g	NZTM2	47.1 (NZMP Wellington)

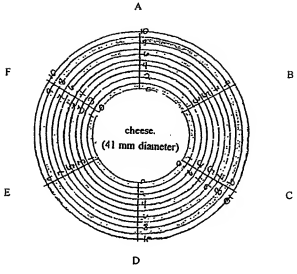
3.6.3 Sensory Evaulation

The cheeses were tested for flavour by a number of informal panels.

3.6.4 Meltability

The cheeses were tested for meltability using the Schreiber and USDA melt tests.

For the Schreiber test, specimens (41 mm diameter) were placed in an oven preheated to 232°C (450°F) for 5 min. A fan-assisted forced-draught oven was used and the Petri dishes were covered. The samples were then removed and cooled for 30 min at room temperature. Expansion was measured along six points (A–F) marked on a concentric set of circles. Meltability was given as the mean of the six readings on an arbitrary scale of 0–10, as shown below.



3.6.5 Shreddability

The processed cheeses were tested to determine their shreddability, which was measured by feeding a block of cheese through a rotating cheese grater. The weight of the cheese block was measured before shredding, and compared with the weight of shred ejected from the grater.

4 Results

4.1 Composition

The compositions of the cheeses produced varied slightly; there was some initial difficulty in getting the pH right when each set of runs was started. The medium fat curds in Run #1 (PT 19517) and Run #2 (PT 19870) differed in composition. The fat content of the Run #2 medium fat curd base was less than the target and the formulations were dosed up with high fat cream. The calcium contents of the two medium fat curd bases differed markedly, because of the fat contents obtained in the final medium fat cheese curds.

4.2 Meltability

All the cheeses showed reasonable meltability, except those made from the medium fat B-flavoured curd base, which had the mould added directly to the processing kettle. The cook temperature of 83°C should have killed all the mould and deactivated any enzymes present. With the mould killed, this should have prevented any proteolysis of the cheese, giving a stable product with more intact casein. Although the compositions of the final cheeses made from the medium fat and low fat curd bases were similar, the meltabilities were vastly different. As the manufacturing process of the medium fat base included homogenisation of the fat, the fat globules within the cheese were small and did not contribute to the meltability of the cheese. The low fat B-flavoured curd base contained large fat globules because the fat source was high fat cream. These large fat globules dispersed within the structure, melted and destabilised the matrix of the curd, contributing to the excellent meltability of the cheese (see Appendix 4).

4.3 Shreddability

The majority of the cheeses were simply too soft, and formed a sticky mat. Only the medium fat base flavour B cheeses (Trials 2 and 8) were suitable, giving 80% shred recovery. Even this cheese produced a somewhat sticky shred that would probably mat together under its own weight, over time. The low fat base flavour B cheeses were much too sticky and soft, despite the similar composition and processing conditions to those of the medium fat base flavour B cheeses. The difference between the two compositions was the fat globule size (see Section 4.2).

Both composite gel curd bases (low fat and medium fat) gave excellent shreddability. The medium fat base was slightly more crumbly than the low fat base (fat globule size).

It is known that a lower moisture cheese curd (< 50% moisture) will shred much more easily than a cheese with greater than 50% moisture. Appendix 3 shows that all the cheeses shredded were > 52% moisture, with some as high as 55% moisture.

Camembert cheese stored frozen at –18°C for around 7 days gave a curd upon thawing that shredded well, irrespective of whether a low fat curd base and high fat cream or a medium fat curd base was used. It was necessary to thaw the frozen Camembert curd for 2–3 h before shredding to obtain good free flowing curd properties. However, it was also noticed that, if the shredded curd was left overnight (5°C), some curd agglomeration occurred.

4.4 Sensory Evaluation

A group of staff associated with natural cheese tasting volunteered to taste the processed cheese samples produced. From the testing, three pleasing flavours were identified. These were:

- **Trial 3: Low Fat Curd Base Flavour A (aged 6 weeks) (5°C Storage Temperature)**
- **Trial 7: Low Fat Curd Base Flavour A (fresh) (5°C Storage Temperature)**
- **Trial 13: Medium Fat Curd Base Whey Protein (both 1 and 6 weeks) (5°C Storage Temperature)**

Other cheeses that were generally well received included:

- **Trial 11: Low Fat Curd Base Flavour C (fresh) (5°C Storage Temperature)**
- **Trial 6: Medium Fat Curd Base Flavour A (fresh) (5°C Storage Temperature)**

From these sensory results, it was clear that adding high fat cream to the low fat composite gel base provided the best flavour. Flavour A was the preferred choice, giving a strong "blue and mushroom" taste to the product.

The instant flavour B cheese was stickier and had more of a processed-cheese-like rubbery texture; most people complained that it lacked flavour and tasted quite acidic.

In a separate trial (13), we added 20% whey protein (ALACEN 392) (based on protein to protein w/w) to the standard medium fat composite gel curd base. This produced a particularly good flavoured cheese with an intense mushroom and creamy flavour – more like traditional Brie or Camembert and different from the flavour of the control sample.

5 Key Findings

- Flavour A with a low fat curd base and high fat cream produced the best flavour (blue + mushroom). The flavour was good for both fresh and aged (6 weeks) cheese.
- Medium fat base with 20% added whey protein and a sprayed surface mould also produced an excellent flavoured (mushroom + creamy) cheese.
- Low fat curd base with high fat cream addition produced superior flavours, but a softer more sticky curd that was unsuitable for shredding after storage at 5°C.
- Both low fat and medium fat curd bases gave excellent shreddability of approximately 96%.
- The cheeses made with medium fat curd base and flavour B gave 80% shred recovery after storage at 5°C. In all other instances, proteolysis of the curd by the mould made it too soft.
- All cheese blocks were easily shreddable when frozen.
- Direct addition of mould into the processing kettle imparted an instant flavour to the cheese.
- The flavour B cheeses made with medium fat curd base did not melt well in the Schreiber oven test. All other cheeses melted well.

6 Discussion

6.4 Instant Flavoured Cheeses

The instant flavour B cheese does not require any ripening time, as the flavour is imparted by the mould added directly into the processing kettle. This reduces storage costs, and any risk of contamination and spoilage of the cheeses. Also, because of the high temperature of the processing kettle, any bacteria and enzymes present in the curd are killed or deactivated and the final product should be very shelf stable.

6.5 Customer Applications

By altering formulations, we could produce a wide range of products for different applications, as follows.

- A shreddable Camembert-flavoured cheese from the medium fat flavour B curd base at 5°C or after frozen storage at –18°C.
- A range of sizes and shapes as the molten curd is poured from the processing kettle.
- A product with an indefinite life under frozen storage.
- Catering applications whereby the cheese can be frozen; there will be no danger of under/overstocking in a restaurant/catering situation.
- Minimum wastage of product.

9 References

Compositional Analysis

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Security Status

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